Malaria in Brazil: what happens outside the Amazonian endemic region

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Brazil, a country of continental proportions, presents three profiles of malaria transmission. The first and most important numerically, occurs inside the Amazon. The Amazon accounts for approximately 60% of the nation's territory and approximately 13% of the Brazilian population. This region hosts 99.5% of the nation's malaria cases, which are predominantly caused by Plasmodium *vivax (i.e., 82% of cases in 2013). The second involves imported malaria, which corresponds to malaria cases acquired outside the region where the individuals live or the diagnosis was made. These cases are imported from endemic regions of Brazil (i.e., the Amazon) or from other countries in South and Central America, Africa and Asia. Imported malaria comprised 89% of the cases found outside the area of active transmission in Brazil in 2013. These cases highlight an important question with respect to both therapeutic and epidemiological issues because patients, especially those with falciparum malaria, arriving in a region where the health professionals may not have experience with the clinical manifestations of malaria and its diagnosis could suffer dramatic consequences associated with a potential delay in treatment. Additionally, because the* Anopheles *vectors exist in most of the country, even a single case of malaria, if not diagnosed and treated immediately, may result in introduced cases, causing outbreaks and even introducing or reintroducing the disease to a non-endemic, receptive region. Cases introduced outside the Amazon usually occur in areas in which malaria was formerly endemic and are transmitted by competent vectors belonging to the subgenus* Nyssorhynchus *(i.e.,* Anopheles darlingi, Anopheles aquasalis *and species of the Albitarsis complex). The third type of transmission accounts for only 0.05% of all cases and is caused by autochthonous malaria in the Atlantic Forest, located primarily along the southeastern Atlantic Coast. They are caused by parasites that seem to be (or to be very close to)* P. vivax *and, in a less extent, by* Plasmodium malariae *and it is transmitted by the bromeliad mosquito* Anopheles (Kerteszia) cruzii. *This paper deals mainly with the two profiles of malaria found outside the Amazon: the imported and ensuing introduced cases and the autochthonous cases. We also provide an update regarding the situation in Brazil and the Brazilian endemic Amazon.*

Key words: malaria - Brazil - *Plasmodium vivax* - extra-Amazon - simian malaria - bromeliads

A short history of malaria in Brazil

At the end of the XIX century, malaria was present throughout the entire Brazilian territory, particularly along the coast. At that time, the Central Plateau and Amazon regions also supported transmission of the disease (Martins Costa 1885, Camargo 2003). The situation remained unchanged, with no epidemic outbreaks, until two important migratory movements occurred in the Amazon. Attracted by the fever of rubber latex extraction and the Madeira Mamoré Road construction, a large number of workers with no previous immunological or cultural experience with *Plasmodium* became exposed to malaria in the Amazon, resulting in an epidemic explosion that caused thousands of deaths (Camargo 2003). According to Chagas (1935), at the end of the 1910s, the main endemic areas in Brazil were the Amazon Region, the São Francisco River Valley and the lowlands of Rio de Janeiro (Baixada Fluminense). At the end of the 1930s, an unexpected, major event occurred in the country: *Anopheles gambiae* was introduced to northeastern Brazil, causing malaria in 80-90% of the population living in the areas invaded by mosquitoes in the state of Rio Grande do Norte (RN) and Ceará (CE) (Deane 1986, 1988). Barros-Barreto (1940) estimated that there were approximately six million cases (i.e., 15% of the total population that year), leading to 80,000 deaths annually in the country in the 1940s. After a successful

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World Health Organization (WHO) malaria eradication campaign was initiated in 1956, the number of cases decreased drastically outside the Brazilian Amazon and the disease reached its lowest level in Brazil, with 36.9 thousand cases in 1960 (Marques & Gutierrez 1994). These figures were slightly augmented in 1970, with 52,469 reports, most of them from the Amazon (Barata 1995, Camargo 2003). Although the colonisation of the Brazilian Amazon effectively began in the 1940s (DDT was used in the region beginning in 1947) (Tauil et al. 1985), it was only in the late 1970s and early 1980s that the intense, rapid and disorganised occupation of the Amazon caused another drastic and important change in the epidemiological situation associated with malaria in Brazil. The Amazon states began to receive large numbers of migrants in search of colonisation projects, environmental resources and economic gains. These migrants came from areas where the disease did not exist or had previously been eliminated. Again, the result was the exposure of a very large number of non-immune individuals to the disease (Barata 1995). Thus, at the end of the 1980s and the middle to end of the 1990s, malaria became the leading health problem associated with communicable diseases in the country (Tauil et al. 1985, Tauil & Daniel-Ribeiro 1998, Oliveira-Ferreira et al. 2010). At that time, over 50% of the total cases registered in Brazil were due to *Plasmodium falciparum* (Fig. 1). Morbidity rates among unprotected, recently settled individuals were extremely high.

In the last 14 years (2000-2013), the Ministry of Health recorded an average of 392.6 thousand cases of malaria per year in Brazil. Unlike the low numbers reported in the mid-1970s, the total number of malaria cases was 615,246 at the beginning of the XXI century (Oliveira-Ferreira et al. 2010). Consequently, a plan to intensify efforts to control malaria [Program for the Intensification of the Malaria Control Actions (PIACM)] was designed and implemented. The main objectives of this plan were to reduce malaria incidence, morbidity (including severe forms) and mortality by adapting control measures to the specific epidemiological conditions of each locality.

Currently, the number of cases of malaria registered in Brazil has been falling yearly and the figures reported in the most recent years are as follows: 267,047 cases in 2011, 242,756 in 2012 (a 9.1% reduction) and 178,613 in 2013 (a 26.4% reduction). An additional 29% reduction in the number of cases was recorded in January-May 2014 compared to January-May 2013[1].

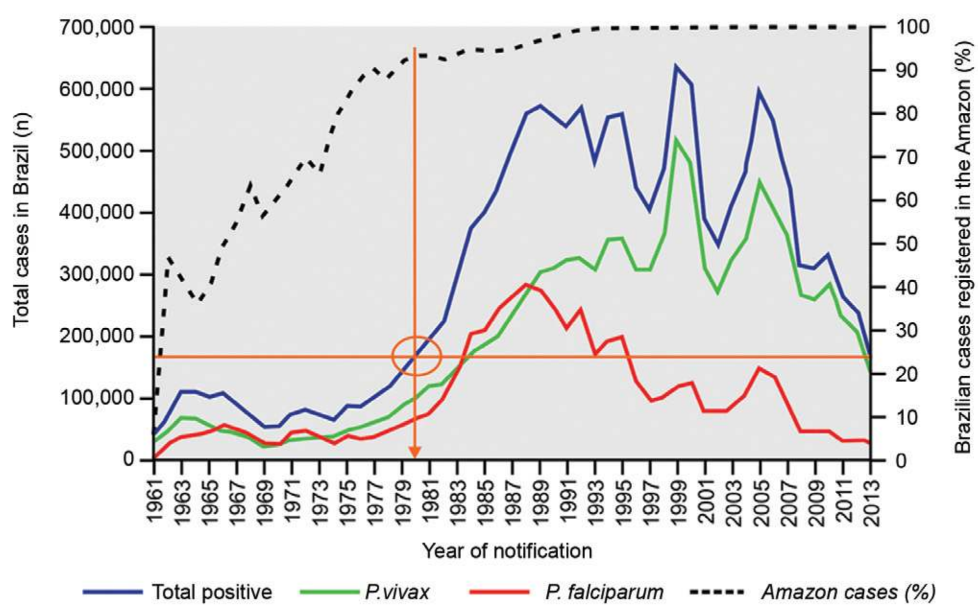

Fig. 1: number of malaria cases registered yearly (1961-2013) in Brazil according to the *Plasmodium* species. After small variations, the low figures recorded in 1961 were slightly augmented in 1970 with most of cases being registered in the Amazon, region that started to concentrate the majority of cases registered in Brazil from 1967 on. The numbers increased steadily thereafter, as a result of the intense, rapid and disorganised colonisation of the Amazon, reaching more than 573 thousand cases in 1989. Two peaks of cases were registered in 1999 and 2005 (around 630 and 600 thousand cases respectively) in spite of a general tendency to decrease the numbers in the last two decades. The circle with the intersection lines shows that the present number of cases corresponds to the figures recorded around 1980, when the percentage of Brazilian cases registered in the Amazon exceeded the 90%. Notice also the progressively decreasing proportion of cases due to *Plasmodium falciparum* since 1988.

[1] The data reported here, that were not derived from scientific publications, were obtained from two databases of the Brazilian Ministry of Health: the Information System for Epidemiological Surveillance - Malaria (SIVEP-Malária) and the Information System for Notifiable Diseases (SINAN). These data have been analysed by the General Coordination of the National Malaria Control Program (PNCM) of the Secretary for Health Surveillance (SVS - ACFSS and MPA). The SIVEP-Malária records malaria cases in the nine states of the Amazonian region and data from 2013 until the end of June 2014 (before the final submission of this paper) were included in this report. The SINAN records data from the other 18 Brazilian states and consists of the extra-Amazon database. As expected, delays in the registration of malaria cases in the SINAN system may upgrade or downgrade some of the data reported in this article; thus, these data cannot be considered as definitive. However, given the magnitude of the numbers, the statistical scenario shown here accurately reflects the reality and trends of malaria transmission and control of malaria in Brazil, including the Amazonian and extra-Amazonian regions.

Morbidity and lethality also decreased from 21,288 hospitalisations and 243 deaths in 2000 to 3,328 hospitalisations and 60 deaths in 2012 and 2,365 hospitalisations and 41 deaths in 2013. This reduction was mainly due to early diagnosis and the prompt treatment policies of the PIACM, which were implemented between 2000-2003 and adopted by the National Malaria Control Program (PNCM) thereafter. In 2013, 40% of the registered cases were diagnosed in the first 24 h and 60% in the first 48 h after the onset of symptoms. Such a strategy, in addition to preventing deaths and the emergence of severe forms of the disease, also decreases the number of cases by reducing the sources of infection and, consequently, the transmission. This is particularly true of *P. falciparum* because gametocytes appear later in the course of this infection. This species accounted for approximately 18% of the cases registered in the country in 2013, while *Plasmodium vivax* was responsible for approximately 82% of the cases (of the total Brazilian cases, including non-autochthonous cases, *P. falciparum* accounted for 16.5%, *P. vivax* and *P. falciparum*, 1.43%, *Plasmodium malariae*, 0.022% and *Plasmodium ovale*, 0.007%).

This scenario may be fundamentally explained by the fact that *P. vivax* has been less responsive to the control interventions implemented by the PNCM in the last 15 years compared with *P. falciparum* due to several unique features: (i) *P. vivax* has a dormant liver stage that can result in relapses even after treatment (White 2011), (ii) *P. vivax* can develop in mosquitoes at lower ambient temperatures than *P. falciparum*, resulting in a greater range of ecological receptivity (Garnham 1966), (iii) unlike *P. falciparum*, *P. vivax* produces infectious gametocytes soon after parasites emerge from the liver and parasite densities are often low (Bousema & Drakeley 2011), and (iv) primaquine, the only drug available to treat the dormant liver stage, requires a long treatment course (7-14 days), which contributes to poor adherence and can result in lower efficacy, as noticed by the PNCM. *P. malariae* is less prioritised than *P. vivax* and *P. falciparum*. The precise burden of *P. malariae* is unknown because identification by microscopy and distinction from blood forms of *P. vivax* may be difficult for a non-experienced examiner who is microscopically inspecting a thick smear (Di Santi et al. 2004) according to PNCM and its identification is not always reliable. In addition, the treatment for vivax malaria is also effective for *P. malariae*.

The Brazilian Amazon Region comprises nine states: Acre (AC), Amapá (AP), Amazonas (AM), Pará (PA), Rondônia (RO), Roraima (RR), Tocantins (TO) and part of the states of Mato Grosso (MT) and Maranhão (MA). According to the Brazilian Institute of Geography and Statistics (IBGE) (ibge.gov.br/cidadesat/link.php), the entire Amazonian region, which has 26.9 million inhabitants (13.4% of the total Brazilian population of 201,032,714 people), accounts for 59.75% of the country's territory of 8,515,767.049 km[2] (IBGE, available from ibge.gov.br/cidadesat/link.php).

A total of 60% of the cases of malaria in South America are derived from the endemic Brazilian Amazon. This region serves as an important source of imported and introduced malaria cases and outbreaks in the extra-Amazonian region of Brazil as well as in other countries (Arévalo-Herrera et al. 2012).

Although malaria transmission is primarily concentrated in the Amazon (99.5% of Brazilian cases in 2013), the distribution of the disease in this region is not homogeneous. Among the 808 Amazonian municipalities, 37 municipalities reported 80.37% of the cases, five municipalities [i.e., Cruzeiro do Sul (AC), Porto Velho (RO), Itaituba (PA), Eirunepé (AM) and Manaus (AM)] reported 30.36% of the cases and three of them [i.e., Cruzeiro do Sul, Porto Velho, Itaituba] reported 21.48% of the total cases in the Amazon (Table I).

The chain of transmission in the region is man-vector-man, with *Anopheles darlingi* serving as the main vector (Oliveira-Ferreira et al. 2010). Endemic malaria in the Brazilian Amazon is classically sustained by *Anopheles* mosquitoes belonging to the subgenus *Nyssorhynchus,* among which *An. darlingi* plays a unique role and is by far the main vector in the Amazon, both inside and outside of Brazil. The expansive Amazon Basin, with its large flood plains and river, provides abundant and suitable larval habitats and favours the development of several *Nyssorhynchus* primary and secondary malaria vectors*,* particularly *An. darlingi* (Deane et al. 1948, Lourenço-de-Oliveira et al. 1989). Despite a few serological and molecular data suggesting that monkeys are a potential reservoir of human malaria in the Amazon (de Arruda 1985, Araújo et al. 2013), there is neither epidemiological nor entomological evidence for natural transmission of simian parasites to humans in this Brazilian region (Lourenço-de-Oliveira & Luz 1996). Therefore, there is no convincing evidence indicating that zoonotic malaria is a threat to disease control in the Amazon.

Malaria transmission is governed by the malaria genesis potential of the area, which is determined by local receptivity and vulnerability. Receptivity is explained by the occurrence of vectors and influenced by several parameters regulating the vectorial capacity of *Anopheles* species, such as density and vector competency and vulnerability is defined by the presence or immigration of gametocyte carriers. Thus, the mitigation of malaria transmission in the endemic Brazilian Amazon will significantly affect the prevention of malaria transmission in both neighbouring countries of Latin America and in extra-Amazonian regions of Brazil, except for the intriguingly residual low malaria transmission in areas under influence of the Atlantic Forest biome, which will be described later herein.

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[2] The PNCM-SVS identifies the Amazonian and extra-Amazonian regions following the SIVEP-Malária/SVS/MS and the SINAN/SVS/MS databases. The SIVEP-Malária screens and records the malaria cases in AC, AP, AM, MA, MT, PA, RO, RR and TO and consists of the data associated with the Amazonian region. Malaria cases reported by MA, MT and TO, which belong only partially to the Legal Amazon, are also recorded in the SIVEP-Malária database, thus integrating the cases of the Amazonian region without distinguishing among the parts of these three states that actually belong to the region.

TABLE I

Municipalities reporting 80% of the malaria cases notified in the Brazilian Amazon in 2013

Municipalities	Municipality	State	Cumulative proportion of cases in the Amazon (%)	Total cases (n)
1	Cruzeiro do Sul	Acre	11.27	20,044
2	Porto Velho	Rondônia	16.41	9,134
3	Itaituba	Pará	21.48	9,004
4	Eirunepé	Amazonas	26.25	8,483
5	Manaus	Amazonas	30.36	7,312
6	Mâncio Lima	Acre	34.46	7,281
7	São Gabriel da Cachoeira	Amazonas	37.56	5,524
8	Ipixuna	Amazonas	40.63	5,454
9	Lábrea	Amazonas	43.25	4,651
10	Atalaia do Norte	Amazonas	45.66	4,291
11	São Paulo de Olivença	Amazonas	48.02	4,190
12	Macapá	Amapá	50.28	4,022
13	Boa Vista	Roraima	52.54	4,011
14	Rodrigues Alves	Acre	54.52	3,524
15	Tabatinga	Amazonas	56.30	3,170
16	Benjamin Constant	Amazonas	58.04	3,091
17	Anajás	Pará	59.73	3,004
18	Tefé	Amazonas	61.36	2,898
19	Guajará	Amazonas	62.80	2,556
20	Jacareacanga	Pará	64.22	2,534
21	Coari	Amazonas	65.65	2,532
22	Alvarães	Amazonas	67.05	2,501
23	Barcelos	Amazonas	68.42	2,423
24	Mazagão	Amapá	69.66	2,217
25	Oiapoque	Amapá	70.91	2,215
26	Calçoene	Amapá	72.10	2,113
27	Itamarati	Amazonas	73.11	1,806
28	Novo Progresso	Pará	74.03	1,635
29	Santana	Amapá	74.91	1,561
30	Uarini	Amazonas	75.72	1,444
31	Tarauacá	Acre	76.49	1,359
32	Santo Antônio do Içá	Amazonas	77.21	1,280
33	Candeias do Jamari	Rondônia	77.93	1,279
34	Jutaí	Amazonas	78.60	1,204
35	Pauini	Amazonas	79.21	1,079
36	Carauari	Amazonas	79.80	1,048
37	Iranduba	Amazonas	80.37	1,020

from 807 Amazonian municipalities, 37 reported 80% of the total number of malaria registered in the Amazon in 2013. Twenty-four out of them responded for 70%, 12 for 50%, five for 30% and only three Amazonian municipalities concentrated 20% of all the Amazonian cases. The 37 Amazonian municipalities with the highest malaria records are located as follows: 21 in the state of Amazonas (56.8%), five in Amapá (13.5%), four in Acre, four in Pará (10.8% each), two in Rondônia (5.4%) and one in Roraima (2.7%).

Malaria in the extra-Amazonian (non-endemic) region

The extra-Amazonian region consists of 18 states: Alagoas (AL), Bahia (BA), CE, Distrito Federal (DF), Espírito Santo (ES), Goiás (GO), Mato Grosso do Sul (MS), Minas Gerais (MG), Paraíba (PB), Paraná (PR), Pernambuco (PE), Piauí (PI), Rio de Janeiro (RJ), RN, Rio Grande do Sul (RS), Santa Catarina (SC), São Paulo (SP) and Sergipe (SE) and parts of the MA, TO and MT[2].

The region covers approximately 40.25% of the Brazilian territory, hosts 86.6% (~174 million people) of the population and has 91.6% of the country's Gross National Product (GNP). In contrast, only approximately 0.5% of the malaria cases registered in Brazil are diagnosed and treated outside the Amazonian endemic region (mean of 1,296 cases/year from 2000-2013) and they present a distinct epidemiological profile. This situation is not comparable in all South American countries that encompass parts of the Amazon Forest in their territories. For example, Colombia has reported the second highest annual number of malaria cases in Latin America (14.2% of all malaria) and most cases (i.e., 90% of malaria cases

TABLE II

Imported and autochthonous malaria cases registered in the extra-Amazon from 2000-2013, according to the macro-regions and states

Regions/states	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013[a]	Total
Southeast Region																			
São Paulo	567	323	363	292	293	269	225	194	231	338	390	263	165	210	247	225	215	174	4,984
Espírito Santo	170	128	102	97	95	74	-	79	157	189	117	93	137	56	80	74	53	56	1,757
Rio de Janeiro	61	50	68	51	72	70	111	77	85	99	119	95	82	84	114	119	133	110	1,600
Minas Gerais	178	208	156	179	187	154	172	114	164	208	145	128	115	86	129	112	106	90	2,631
Subtotal	976	709	689	619	647	567	508	464	637	834	771	579	499	436	570	530	507	430	10,972
South Region																			
Paraná	455	379	252	324	204	146	225	163	166	206	161	204	97	121	120	1045	61	64	3,452
Santa Catarina	59	34	46	27	50	42	-	49	496	54	45	40	16	17	36	23	46	41	674
Rio Grande do Sul	38	37	28	26	36	19	12	19	35	27	33	17	14	11	25	18	17	13	425
Subtotal	552	450	326	377	290	207	237	231	250	287	239	261	127	149	181	145	124	118	4,551
Midwest Region																			
Goiás	364	243	363	290	160	52	137	173	190	176	107	115	72	64	129	71	79	60	2,845
Distrito Federal	117	97	109	76	90	59	58	62	58	59	58	41	25	29	53	40	28	25	1,084
Mato Grosso do Sul	111	86	94	63	238	233	90	60	59	85	72	40	34	34	28	30	26	21	1,404
Subtotal	592	426	566	429	488	344	285	295	307	320	237	196	131	127	210	141	133	106	5,333
Northeast Region																			
Alagoas	9	9	10	11	12	0	12	5	9	6	4	6	6	10	9	6	7	8	139
Bahia	121	55	47	53	70	96	42	90	33	42	31	33	27	21	64	26	20	20	891
Ceará	102	79	68	90	135	64	464	38	67	71	54	67	33	34	55	33	31	19	1,504
Paraíba	14	14	16	13	20	2	3	5	5	12	5	2	0	9	19	7	9	7	162
Pernambuco	61	41	27	46	36	19	9	11	24	21	8	28	38	34	26	30	20	24	503
Piauí	134	129	132	159	277	133	64	85	147	65	100	54	57	60	118	109	71	80	1,974
Rio Grande do Norte	14	23	30	25	24	22	13	10	9	17	17	4	7	14	18	19	22	10	298
Sergipe	8	8	1	6	7	5	0	9	4	7	2	2	3	4	7	7	6	5	91
Subtotal	463	358	331	403	581	341	607	253	298	241	221	196	171	186	316	237	186	173	5,562
Total	2,583	1,943	1,912	1,828	2,006	1,459	1,637	1,243	1,492	1,682	1,468	1,232	928	898	1,277	1,053	950	827	26,418

a: see footnote 1. Most of the malaria cases registered in the extra-Amazon from 2000-2013 occurred in the Southeast Region (41.6%). The state with the highest number of cases in this period was São Paulo (20.2%), with more cases than all of the South Region states together (17.2%). In the Southern Region, Paraná had more cases than the others together (3,452). In the Midwest Region (20.1%), the predominance of cases happened in Goiás. In the Northeast Region (21%) the predominance of cases happened in Piauí.

ceptive area (with competent vectors). An autochthonous case occurs in a location where there is a source of infection. As described in the Atlantic Forest section of this article, this category of malaria may occur as a zoonosis, involving non-human reservoirs and competent vectors. A special variety of malaria known as "airport malaria" occurs when infectious mosquitoes from endemic areas are introduced in a new, malaria-free region and feed on the blood of local residents, usually in the airport neighbourhood, causing an outbreak of the disease. We will not address the "airport malaria" in this paper.

Some cases of malaria are imported from endemic areas inside or outside Brazil - The number of malaria cases in travellers has been steadily increasing over the past three years (Centers for Disease Control and Prevention, available from: nc.cdc.gov/travel/yellowbook/2014/chapter-5-post-travel-evaluation/general-approach-to-the-returned-traveler).

Travel-related health problems have been reported in as many as 22-64% of individuals travelling to developing countries. Malaria should always lead the list in the differential diagnosis of fever, as well as fatigue and headaches, in travellers or migrants who have been in an endemic area within the previous few months; this is the case even if they have taken medication to prevent malaria because prophylaxis may not be 100% effective and patients may sometimes miss doses of the medication. Clinicians should have a low threshold for admitting febrile patients if malaria is suspected. Because of the ability of *P. falciparum* infection to progress in just a few hours to severe and life-threatening complications, it is advisable to hospitalise any non-immune individual during their initial period of treatment. Physicians must be alert to recognising and treating malaria to avoid severe morbidity or a fatal outcome (Fairhurst & Wellems 2009). The lethality rate of imported malaria caused by *P. falciparum* is 4%, which is 30-40 times higher than that in patients with uncomplicated malaria, but five-six times lower than that of patients with vital organ dysfunction (e.g., cerebral malaria) (White & Breman 2005).

The recent economic boom in Brazil has led to an increase in construction and development by Brazilian mining and oil exploration companies in endemic regions of the country as well as in the African continent. The changing pattern of imported malaria in unaffected states already reflects this phenomenon. In SP and RJ, for instance, the increasing number of suspected malaria cases imported from Africa may reflect these migratory movements (Lupi et al. 2014, SES-SP/CCD/CVE 2014).

Most of the cases (739 out of 827 or 89.3%, in 2013) diagnosed and reported outside the Brazilian Amazon corresponded to imported cases originating from the Amazon (376 cases, 50.9% of the total imported cases) or other Central and South American, African or Asian countries that have active transmission (363 cases, 49.1%). One indicator of the different sources of malaria cases is the higher proportion of falciparum malaria in the extra-Amazonian region; 36% of the cases registered in 2013 (mean 31.7% from 2007-2013) were falciparum malaria.

Due to the low incidence of the disease, diagnosing malaria in this region is a major challenge and requires clinical and laboratory personnel trained in recognising this disease and able to make an accurate laboratory diagnosis. Practically, this may indeed be difficult in places where the disease is not part of the routine experience of the local doctors. For example, it has been estimated that in Rio de Janeiro, the probability of identifying a case of malaria (of which there are approximately 150/year) is 26 times lower than the probability of diagnosing a case of acute leukaemia (4,000/year) and 466 times lower than the probability of identifying a case of dengue fever (70,000/year) in the emergency department of a large general hospital. In dengue epidemic years, the probability of identifying an individual with malaria may be 2,700 times lower than the probability of identifying a dengue fever patient (O Lupi, unpublished observations). It is worth noting that 55% of the malaria cases examined at the Evandro Chagas National Institute of Infectology-Oswaldo Cruz Foundation, a reference centre for diagnosis and treatment in RJ, were clinically mistaken for dengue fever during the first encounter with care from the support network of the city of Rio de Janeiro (P Brasil, unpublished observations). In addition, 14% of the vivax malaria patients examined at the same Centre between January 2005-February 2010 who acquired the disease in the Brazilian Amazon presented an incubation period of over 90 days (up to 130 days) in the absence of any chemoprophylaxis (Brasil et al. 2011); this situation may render the diagnosis even more difficult in patients who must recall and report a stay in an endemic area that may relate to the present disease.

Only 19% of all extra-Amazonian cases of malaria are diagnosed and treated in the first 48 h after the onset of symptoms, contrasting with 60% in the Amazon. This may explain the greater proportion of severe cases. The malaria lethality rate is thus much higher in the extra-Amazonian region than in the Amazon Region. Between 1996-2013, there was a 187.2% increase in the proportion of deaths in the extra-Amazonian region. The lowest death rate was registered in 2004 (0.47%) and the highest death rate was registered in 2009 (1.78%), representing a 380% increase in the risk of death. The coefficient of lethality in the extra-Amazonian region was approximately 90 times higher than that recorded in the Amazon Region in 2011, approximately 40 times higher in 2012 and approximately 60 times higher in 2013 (data subject to revision at the Mortality Information System bank, Secretary for Health Surveillance - SVS). Considering only falciparum malaria cases, the coefficient of lethality was 72, 30 and 40 times higher in the extra-Amazonian region compared with the Amazon Region in the same years. Practically, this indicates that the chance of dying from malaria is tens of times higher if the disease is diagnosed outside the Amazon, even in states that have much more advanced technological and medical resources than those available in the Amazon, which has less than 9% of the country's GNP.

Only a small fraction of malaria in Brazil corresponds to autochthonous cases registered outside the Amazon endemic region - A total of 932 autochthonous cases were registered in the extra-Amazonian region between 2007-2013. It is quite probable that a few of the cases described as autochthonous correspond, in reality, to introduced cases that are secondary to imported cases. In fact, distinct categories are not available for differentiating autochthonous from introduced cases in the extra-Amazonian region in the registration system of the SVS. The difference is noted in the epidemiological survey each time an outbreak is reported. Although it is quite probable that a case reported during an outbreak in a non-endemic region corresponds to an introduced case, in some instances, this case may not be linked to the index case, and it may be considered autochthonous.

Of the 827 cases registered in 2013 in the extra-Amazonian region, only 10.6% (88 cases) were due to autochthonous transmission; this corresponded to only 0.05% of the total cases in Brazil. In 2012, 10.4% of cases in the extra-Amazonian region (99 out of 950 cases) were autochthonous, which corresponded to 0.04% of all Brazilian cases.

The population of the southeastern region is over 84 million, accounting for 42% of the total Brazilian population (IBGE, available from ibge.gov.br/cidadesat/link.php). Four Brazilian states (SP, RJ, ES and MG) out of a total of 18, including DF, contained 57.1% of all 932 autochthonous cases registered in the extra-Amazonian region in the past seven years. Of all the cases, 34.4% were concentrated in ES (321 cases), 18.2% were in SP (170 cases), 3.8% were in RJ (35 cases) and 0.6% were in MG (6 cases).

Including PR (17.6%, 164 cases), which is located in the South Region, the four states from the southeastern region reported 74.7% of the autochthonous cases diagnosed between 2007-2013. The other autochthonous cases (236) registered during this period occurred in PI (98 cases), GO (35 cases), MS (31 cases), BA (26 cases), PE (18 cases), RN (9 cases), CE (7 cases), DF (5 cases), RS (4 cases), SC (2 cases) and AL (1 case). In SC, in the period from 1996-2001, 84 autochthonous cases (Machado et al. 2003) were identified.

Of the 96 autochthonous cases registered in known municipalities of the extra-Amazonian region in 2012, 77 (80.2%) were recorded in areas covered by the Atlantic Forest domain. In addition, the 96 autochthonous cases registered in 2012 occurred in 43 municipalities, 26 (60.5%) of which are located under the Neotropical Atlantic Forest domain (mapas.sosma.org.br). Therefore, it is possible that at least some of the remaining cases in areas outside the Atlantic Forest correspond, in reality, to introduced cases or cases secondary to imported cases.

Indeed, as discussed in the section *The entomological aspects of malaria transmission outside the Amazon*, cases classified as autochthonous in the extra-Amazon region can be didactically grouped into two distinct epidemiological situations: (i) Cases or outbreaks occurring in formerly malaria endemic plains, lowlands and plateaus generally correspond to introduced cases derived from imported cases in receptive areas; these imported index cases have usually, but not necessarily, been identified. They are caused by *Plasmodium* that exhibits traditional clinical "behaviour" with respect to parasitaemia; they are transmitted by *Anopheles* of the subgenus *Nyssorhynchus* (*An. darlingi, Anopheles aquasalis* or *Anopheles* of the Albitarsis complex) and their transmission ceases due to transmission blocking actions triggered by the presence of the case(s). (ii) Cases or outbreaks occurring in mountain valleys are usually non-introduced autochthonous cases caused by a *Plasmodium* that is morphologically similar to *P. vivax* or *P. malariae*. In those areas, *Plasmodium* parasite transmission is associated with *Kerteszia* mosquitoes and does not depend on any previously detected imported index human case. The low transmission has a cyclic pattern, with a higher number of cases in the summer and the transmission resists any blocking actions executed by the surveillance programs.

Examples are mentioned in Table III and include the following. The most important epidemic in the extra-Amazonian region in recent years certainly occurred in

TABLE III

Outbreaks of malaria in the extra-Amazon region, Brazil, from 1996-2013

State	Year	Cases[a] (n)	Reference	Identification of the index case
Santa Catarina	1996-2001	84	Machado et al. (2003)	ND
Minas Gerais	1980-1992	471	Chaves et al. (1995)	ND
São Paulo	1981	9	Andrade et al. (1986)	ND
	1984	10		ND
Ceará	2002	402[b]	SES/Ceará	An imported case from Nova Esperança do Piriá, Pará, Brazilian Amazon
Piauí	2004	109	Chagas et al. (2005)	Undetermined number of imported cases from Suriname
	2010	26	MFB Chagas, unpublished observations	ND
	2011	5	MFB Chagas, unpublished observations	An imported case from Maranhão, Brazilian Amazon
	2013	14	SES/Piauí	An imported case from Guiana

a: introduced and autochthonous cases; *b*: more four cases are being investigated; ND: not determined; SES: State Secretary of Health.

2002 in CE, with 402 cases due to *P. vivax* occurring over a 30-week period. The index case (i.e., a case imported from Nova Esperança do Piriá, PA, and registered at Córrego dos Cavalos, municipality of Marcos, a small city in northern CE along the Tucunduba River on the border of Senador Sá county) reintroduced vivax malaria transmission to the locality of Córrego dos Jenipapos, northern CE, where the index case remained for a week. According to the Secretary of Health in this locality, this index case interrupted the harmonious coexistence between man and *An. darlingi*, predominantly at the natural breeding sites on the Tucunduba River.

In MG, 471 cases of autochthonous malaria were detected in 29 foci of the state from 1980-1992 with a mean of 38 cases per year (Chaves et al. 1995). From 2007-2013, six autochthonous cases were reported in the state (PNCM); however, there is no published information regarding these cases in the scientific literature.

Other epidemic foci, most likely also resulting from imported cases, have occurred in recent years (from 2007-2013): one in AL, 26 in BA, seven in CE, five in DF, 35 in GO, 31 in MS, 18 in PE, 98 in PI, 164 in PR, nine in RN, four in RS and two in SC.

In the western region of SP in the 1970s, transmission was associated with the Paraná River's high-water season; the last autochthonous case was reported in 1993 (SUCEN/SES-SP 1995, Gomes et al. 2008). *P. falciparum* was responsible for two important outbreaks in the 1980s, with nine cases reported in 1981 in the region of São José do Rio Preto and 10 cases in 1984 in Panorama; both outbreaks were in the western region (Andrade et al. 1986).

Other illustrative situations have occurred in PI, which is located in northeastern Brazil since 2004 (Table III). In 2013, one imported case from Guiana originated an outbreak with 14 cases of *P. vivax* malaria confirmed by laboratory diagnosis. At least 10 cases were reported in Campo Largo do Piauí in only 20 days (20 May-12 June), the first case being a young boy who had accompanied his family on a fishing trip to a lagoon (i.e., Lagoa do Projeto), where he stayed until 06:00 pm. At the same time, one additional case was registered in the municipality of Porto (PI).

Geographic and environmental aspects of the Atlantic Forest - The characteristics of the Atlantic rainforest create ecological conditions suitable for reproduction of some mosquito vector species. Forests with bromeliad cover provide a favourable habitat for *Kerteszia* vectors (Deane 1992, Guimarães et al. 2003, Domingos et al. 2006).

The Atlantic rainforest is considered to be a world biosphere reserve. The wide range of altitudinal and latitudinal conditions and the complex topography influence temperatures and rainfall distribution, resulting in a highly diverse climate and a large amount of biological diversity and endemism; thus, many species of animals and plants are unique to this region (Ribeiro et al. 2009).

Originally, the Atlantic Forest was a terrestrial biome, which extended along the Atlantic Coast of Brazil from RN to the north to RS, reaching parts of southeastern Paraguay and northern Argentina. This forest occupied tropical and subtropical regions in 17 states in Brazil (i.e., all the extra-Amazonian states, except MT and DF). Before European occupation, the Atlantic Forest covered a total area of 1,300,000 km², representing 15% of the country (Ribeiro et al. 2009). Presently, only 7-8% of the forest still exists (IBGE, available from mapas.sosma.org.br) (Fig. 2).

Approximately 118 million people (62% of the Brazilian population) live in regions under the influence of the Atlantic Forest, which is submitted to continuous pressure because part of the forest has been impacted by human activities (Ribeiro et al. 2009, Fundação SOS Mata Atlântica, available from: sosma.org.br/nossa-causa/a-mata-atlantica/2014). The climate of the Atlantic rainforest is hot and humid, which is typical of tropical and subtropical rainy zones (Köppen Climate System). It has high average temperatures, cloudy highlands and high humidity; the rainy season occurs in southern and southeastern (SE) Brazil from November-March and the dry season occurs from May-September (IBGE, available from ibge.gov.br/cidadesat/link.php). In northeastern Brazil, the rainy season lasts approximately from March-June. Average annual rainfall ranges from 900 mm in the north to 2,600 mm on the slopes of the highlands. The rainfall is evenly distributed with very wet summers and dry winters.

The Atlantic Forest is divided into eight biogeographical sub-regions (i.e., Araucaria, BA, Brejos Nordestinos, Diamantina, Interior, PE, Serra do Mar and São Francisco Forest) in 17 Brazilian states (Silva & Casteleti 2003). Small isolated fragments of less than 50 ha, covered by second-growth forest, compose 83% of the Atlantic Forest. Only 13% of the forest lies within the three largest fragments located in the Serra do Mar. One is between SP and RJ, another is in the coastal zone of SC and the third is located in the coastal zone of PR. These large fragments exist in areas where human occupation is difficult (Silva et al. 2007).

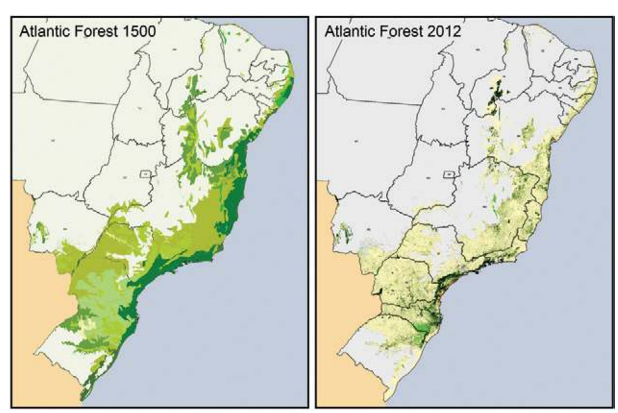

Fig. 2: Brazilian territorial surface occupied by the Atlantic Forest: map comparing the extension of the area covered by vegetation in 1500 and in 2012. Originally, the Atlantic Forest extended along the Atlantic Coast of Brazil from the state of Rio Grande do Norte, in the Northeast Region, to the north to Rio Grande do Sul, in the South Region, reaching parts of southeastern Paraguay and northern Argentina. Before the European occupation, the Atlantic Forest covered a total area of 1,300,000 km², representing 15% of the country's territory. Presently, only 7-8% of the residue forest still exists (Fundação SOS Mata Atlântica/INPE 2008).

Serra do Mar, where most malaria cases occur, is the best preserved sub-region. The topography is characterised by highlands (*serras*) with large continuous forest preserves, peaks at approximately 3,000 m above sea level (ex. Pico das Agulhas Negras) and lowlands (*baixadas*) at sea level with intermixed mosaics containing spaces of deforested areas, human occupation and changes in land use (Ribeiro et al. 2009). The wide range of altitudinal and latitudinal conditions, as well as the complex topography influence the temperature and the rainfall distribution, resulting in a highly diverse climate and wide biological diversity.

Malaria in the Atlantic Forest - Of the 26 Atlantic Forest municipalities with registered autochthonous cases in 2012, 18 (69.2%) were located in SP, ES, RJ and MG, which compose the Southeast Region; these cases accounted for 68 (88.3%) of the cases in the Atlantic Forest domain. Except MG, all the states in the Southeast Region are located along the coast. The remaining 30.8% of the Atlantic Forest municipalities harbouring malaria cases were located in BA, CE, MS, PI and RN.

In ES, malaria cases have been recorded since 1976 in highlands located no more than 50 km from the Atlantic Ocean. Autochthonous cases have been reported each year and many of them have been reported in nine municipalities distributed over an area of approximately 5,343 km^2 with a population of 215,000 inhabitants (Cerutti 2007, Cerutti et al. 2007). In 2013, ES reported 37 autochthonous cases. Diagnosis based on haemoscopy revealed only the presence of *P. vivax* with very low parasitaemia; however, polymerase chain reaction (PCR) showed 0.9% positivity for *P. malariae*. Additionally, the serological profile of the population suggests a high exposure rate to *Plasmodium* because indirect fluorescent antibody assays showed a positivity of 37.7% for *P. vivax* and 7.9% for *P. malariae*; this may indicate that individuals spontaneously clear the infections without seeking treatment due to the atypical clinical features of infection in the area (Cerutti et al. 2007). Cerutti et al. (2007) postulated two hypotheses regarding the source of infection in this region; the first highlights the large number of asymptomatic cases and the second considers simian reservoirs for the parasites, which may act as a source of infection for humans.

Transmission of autochthonous malaria in SP is characterised by sporadic outbreaks in the western region (Andrade et al. 1986) and persistent transmission in the eastern region, which is home to the Atlantic Forest biome; here, oligosymptomatic or even asymptomatic cases occur with low levels of parasitaemia caused by *P. vivax* (Carvalho et al. 1985, 1988, Barata 1995, Branquinho et al. 1997, Couto et al. 2010) and *P. malariae* (Scuracchio et al. 2011). The primary vector in the western region is *Anopheles* of the subgenus *Nyssorhynchus*, whereas in the eastern region, transmission occurs mainly due to *Anopheles (Kerteszia) cruzii*. In a study that analysed data from 1980-2007, 821 autochthonous cases were reported; 91.6% were in the eastern region and most of them (97.2%) were due to *P. vivax* according to haemoscopy. *P. falciparum*, *P. malariae* and mixed infections were identified in 14, five and three patients, respectively. The municipalities with the highest numbers of malaria cases were Peruíbe (135), Juquitiba (81), São Paulo (65), São Sebastião (58), Miracatu (44), Iporanga (36), Pedro de Toledo (36) and Sete Barras (35), which are all located in the eastern region in the Atlantic Forest biome. In the western region, 14 autochthonous cases were registered in Araçatuba (Palmeira d'Oeste, 9, Presidente Epitácio, 6, Teodoro Sampaio, 5, and Castilho, 5). It is noteworthy that 9.6% of these 821 locally acquired infections were asymptomatic and 80.7% presented low parasitaemia. Asymptomatic carriers were detected in surveillance activities during outbreaks or in surveys related to studies in the Atlantic Forest. The incidence rates of malaria in SP from 1980-2007 show a decreasing trend (Couto et al. 2010).

In RJ, autochthonous cases have been occurring annually since 1993 in the region of the Lumiar, district of Nova Friburgo, a mountainous touristic region that receives many visitors each year; some of them come from the Amazon on pilgrimages to the Santo Daime Lumiar and São Pedro da Serra temples. These areas also receive trucks of wood from the Amazon. Malaria cases have been primarily detected among visitors that visit the forest in the slopes (Mattos et al. 1993, Azevedo 1997, Costa et al. 2009, 2010, Veltri et al. 2011, Brasil et al. 2013). Other cases began to be described in 2008 in Guapimirim, which is also a touristic region near the Atlantic Forest, in the localities of Garrafão and Monte Olivette, which are close to the forest with altitudes ranging from 340-730 m. Since 2011, cases have been identified in Sana, a district of Macaé. Sana is also located in a mountainous area and is also a touristic region. However, differently from the Nova Friburgo, the Sana cases occurred near virgin forest with altitudes ranging between 335-1,004 m and far from the touristic area. Bromeliads are abundant in all areas and the vector incriminated in the transmission of the Nova Friburgo and Sana cases was *An. (Ker.) cruzii* (T Silva-do-Nascimento, unpublished observations). Other isolated cases have also occurred in the municipalities of Cachoeiras de Macacu, Teresópolis and Sapucaia, located at about 100, 95 and 145 Km far from the state capital.

Differences in the prevalence of autochthonous malaria between the states in the extra-Amazonian region may result from failures in the local notification processes. Asymptomatic infections reported in the Atlantic Forest area represent an enormous challenge for malaria control. One of the most important challenges is that concerning the risk of transfusional malaria transmission in the extra-Amazonian region (Maselli et al. 2014). For example, *P. malariae* was identified by PCR in a case of transfusional malaria that caused the death of the recipient, an individual who had been submitted to splenectomy and was immunocompromised. The patient received infected blood from an asymptomatic donor who had visited Iguape on the coast of SP two years before the donation. Another recipient, who received blood from the same donor, was also diagnosed with *P. malariae* and remained asymptomatic, harbouring parasites in the peripheral blood that were detected only by PCR (Kirchgatter et al. 2005). Two other cases were detected from donors infected in Juquitiba (Di Santi et al. 2005) and Juquiá (Scuracchio et al. 2011), which are two municipalities located in the Atlantic Forest area. In Rio de Janeiro, two cases of *P. malariae* malaria were likely also related to blood transfusions following orthopaedic surgery in a private clinic; the blood bank did not identify infected donors from Atlantic Forest villages (P Brasil, unpublished observations). Also using PCR analysis, Maselli et al. (2014) detected *P. falciparum* and *P. vivax* carrying asymptomatic individuals among 5.1% and 2.3% of healthy blood donors at the Pro-Blood Foundation/Blood Centre of São Paulo, the main blood transfusion centre in São Paulo. Positive individuals came from the Atlantic Forest or near it.

As a rule, serological assays are not useful for the diagnosis of an acute case of malaria; however, they may be important for indicating or estimating the exposure of individuals to malaria antigens. Despite the low number of autochthonous clinical malaria cases registered in the extra-Amazonian region, the results from serological studies in areas covered by the Atlantic Forest biome indicate that inhabitants of most of these areas produce antibodies against asexual forms of *P. vivax* and *P. malariae*, with prevalence as high as 32-49% and 16-19.3%, respectively. Additionally, they produce antibodies against the circumsporozoite of *P. vivax* and the variants *P. malariae* and *P. falciparum* (Carvalho et al. 1988, Mattos et al. 1993, Azevedo 1997, Curado et al. 1997, 2006, Duarte et al. 2006, Cerutti et al. 2007, Yamasaki et al. 2011, Neves et al. 2013). A study performed using PCR as a diagnostic tool to detect *Plasmodium* infection in human populations of Vale do Ribeira identified individuals without classical symptomatology who were infected with *P. malariae*, *P. falciparum*, *P. vivax* and *P. falciparum*/*P. vivax* (Curado et al. 2006). The authors hypothesised that asymptomatic individuals may act as a source of transmission in the extra-Amazonian region.

The entomological aspects of malaria transmission outside the Amazon - Although most cases reported in the country have been registered in the Amazon Region, the conditions throughout nearly the entire territory of Brazil are suitable for malaria transmission.

Approximately 60 anopheline species have been identified in Brazil and primary or secondary human malaria vectors have been recorded in all states, which means that they are receptive to malaria transmission. The anopheline vector species implicated in malaria transmission outside the Amazon vary according to environmental and epidemiological situations. Accordingly, two major situations have been recorded outside the Amazon: introduced cases and/or outbreaks erupting on inland plains, plateaus, gently undulating terrains (< 20% slope) or coastal lowlands, where malaria is transmitted by *Anopheles* belonging to the subgenus *Nyssorhynchus* and autochthonous and low-transmission malaria occurring in the southeastern mountain valleys, where the infection is transmitted by *Anopheles* belonging to the subgenus *Kerteszia* (Deane 1986, Meneguzzi et al. 2009).

The large river basins that were once traditional endemic malaria zones located on plains, plateaus and gently undulating lands are still receptive to transmission due to the existence of perennially suitable larval habitats (e.g., backwaters, dams, large flooded areas) for *Nyssorhynchus,* particularly *An. darlingi,* which is the major South American malaria vector. This is the case with most of the territory outside the Amazon, comprising the south Amazon (part of MT and TO), northeast-Atlantic (part of MA and PI), Parana-Paraguay (MS, SP, part of MG, PR, SC and RS), São Francisco Basin (part of MG, BA, PB, SE and AL) and to some extent the eastern-Atlantic Basin (e.g., Rio Doce Basin, RJ, ES and BA in part), where *An. darlingi* doubtless plays a key role in malaria transmission by being the most anthropophilic and most common (or only) anopheline that bites humans indoors and in the vicinity of dwellings. The numerous water reservoirs from recently constructed hydroelectric plants increased the number of suitable larval habitats for *An. darlingi* as well as for other potential *Nyssorhynchus* malaria vectors, such as species of the Albitarsis and Triannulatus complexes (Gomes et al. 2008, 2013, Limongi et al. 2008, Meneguzzi et al. 2009, Da Silva et al. 2013, McKeon et al. 2013, Ribeiro et al. 2013). Along the coastal lowlands, introduced malaria cases and/or small epidemics have emerged on occasion and localities with brackish water and *Nyssorhynchus* and *An. (Nys.) aquasalis* are abundant. These conditions have supported sporadically introduced malaria cases and outbreaks in the coastal lowlands of RJ, ES and CE. *An. (Nys.) aquasalis* occurs along almost the entire Brazilian Coast except southern SP, PR, SC and RS, rendering all these extra-Amazonian territories receptive to malaria introduction when their populations peak (Deane et al. 1948, Flores-Mendoza & Lourenço-de-Oliveira 1996, Meneguzzi et al. 2009).

In fact, introduced malaria in the extra-Amazonian region of Brazil primarily emerges where endemic malaria due to transmission by *Nyssorhynchus,* particularly *An. darlingi*, has occurred in the past (i.e., up to the 1960s). Despite the sanitary improvements and large environmental changes that have occurred in this Brazilian region since then, most of the territory remains highly receptive to malaria transmission. The degree of receptivity and vulnerability depend on the local anopheline fauna composition and density, as well as the intensity of human displacement from endemic areas, respectively.

Unfortunately, confirmation of the *Nyssorhynchus* anopheline species involved in the transmission of introduced malaria cases and outbreaks in the inland plains, plateaus and coastal lowlands has rarely been undertaken. Entomological surveys conducted following the discovery of a single malaria case usually involve simply recording the composition of anopheline fauna and highlighting the most abundant species; these surveys seldom note the most frequent species biting indoors and local entomological teams cannot search for natural plasmodia infections in mosquitoes. Therefore, the determination of malaria vectors outside the Amazon has mostly been based on behavioural and biological data gathered during and just after the appearance of a newly introduced case or outbreak.

Fig. 3: *Anopheles (Kerteszia) cruzii*, the mosquito vector of both the human and simian malarias in the Atlantic Forest of southern and southeast Brazil. Photo by Genilton Vieira.

Fig. 4: the malaria vector *Anopheles (Kerteszia) cruzii* breeds in water accumulated in the axils of shaded and epiphyte bromeliads, such as *Vriesea* sp. Photo by Genilton Vieira.

Concerning the autochthonous and low-transmission malaria occurring in the southeastern mountain valleys, several recent efforts have been made to clarify ecological and entomological characteristics of this particular epidemiological scenario, in which *Anopheles* belonging to subgenus *Kerteszia*, particularly *An. (Ker.) cruzii,* play a primary vectorial role (Lorenz et al. 2012) (Fig. 3). However, most determinants related to the generation of new cases and small outbreaks from an essentially silent transmission cycle remain unknown. In the mountain valleys, the slopes (generally > 20%) prevent water from being retained on the ground surface; thus, larval habitats suitable for *Nyssorhynchus* are rare or absent. Those that do arise are usually flushed during the rainy season, killing immature life forms. Thus, water held in tanks at the base of bromeliad leaves provides a suitable larval habitat for several mosquito species, including anophelines of the subgenus *Kerteszia.* In the case of *An. cruzii*, shade and epiphytic bromeliads are the preferred host plants, although hundreds of bromeliad species have been described as habitats for this mosquito species in the Atlantic rainforest, as those belonging to genus *Vriesea* (Fig. 4). The Atlantic Forest covering the Serra do Mar hills and valleys is particularly rich in bromeliads and provides a highly suitable habitat for *An. cruzii* and other *Kerteszia* species (Aragão 1968, Laporta et al. 2011). *An. cruzii* is considered to be a species complex (Carvalho-Pinto & Lourenço-de-Oliveira 2004, Rona et al. 2013) and its vectorial competence has not yet been determined. Regardless, *An. cruzii s.l.* has been shown to be the most important or - depending on the condition - the sole primary vector of so-called "bromeliad malaria" in southern and southeastern Brazil for decades (Forattini 1962, Aragão 1968, Deane 1986, Duarte et al. 2013). *An. cruzii* is an acrodendophilic mosquito; however, depending on the area and environmental/climatic situation, it may bite almost exclusively in the forest canopy or attack both at the canopy and at ground level (Fig. 5). In the latter situation, *An. cruzii* has an important role in the low-level transmission of autochthonous malaria in the southeastern mountain valleys because it may bite humans and non-human primates with similar frequencies (Deane 1986, Azevedo 1997, Cerutti 2007, Ueno et al. 2007, Duarte et al. 2008, Yamasaki et al. 2011). In these southeastern mountain valleys, *An. cruzii* is by far the most frequent species that bites humans in wilderness, transition and modified areas and almost the only species found to naturally carry *P. vivax*/*Plasmodium simium* and *P. malariae/Plasmodium brasilianum* (Curado et al. 1997, Marques et al. 2008, Rezende et al. 2009, 2013, Duarte et al. 2013, Neves et al. 2013).

Malaria in extra-Amazonian mountain valleys may be a zoonosis - At least 26 *Plasmodium* species are known to infect primates (Kantele & Jokiranta 2011). Until recently, the natural transmission of a non-human *Plasmodium* species to humans was considered to be rare or accidental (Deane 1992, Ta et al. 2014). However, in 2004, an outbreak of human infections with the simian parasite *Plasmodium knowlesi* from non-human reservoirs was confirmed in Malaysia and Southeast Asia (Cox-Singh et al. 2008). *P. knowlesi* was previously considered a non-human primate parasite. However, it was discovered that *P. knowlesi* was misdiagnosed as *P. malariae* by direct examination of blood films of human Malaysian malaria cases, as the diagnosis of these cases was only possible by molecular tests. Therefore, *P. knowlesi* is currently considered by many to be a fifth *Plasmodium* species that naturally infects humans (Cox-Singh et al. 2008). Some authors, however, will not consider *P. knowlesi* as a human parasite until natural human-to-human transmission is demonstrated. Following these first cases, imported human cases due to *P. knowlesi* have been reported in many other Asian countries (Jongwutiwes et al. 2004, Luchavez et al. 2008, Ng et al. 2008, Van den Eede et al. 2009, Cox-Singh 2009, Jiang et al. 2010, Khim et al.

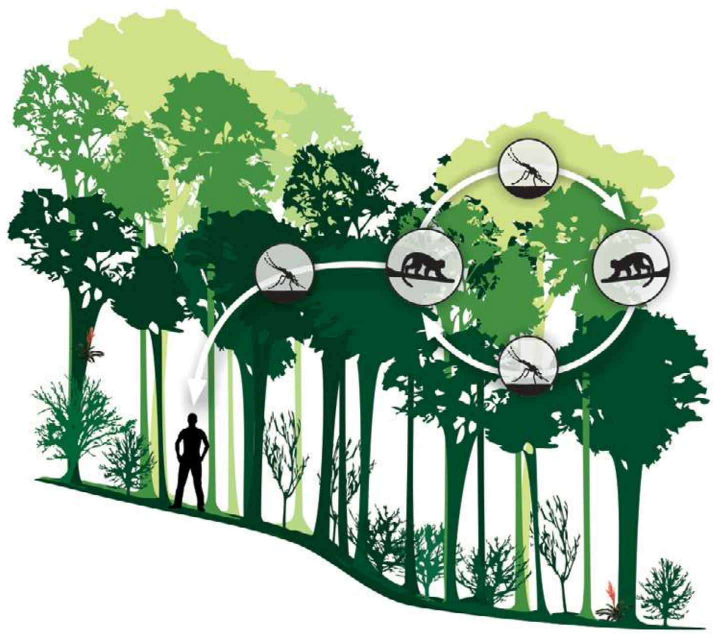

Fig. 5: in some conditions, *Anopheles (Kerteszia) cruzii* may bite both at the canopy of the trees and close to the ground, which may favour the transmission of simian plasmodia to human in the wild or in the close vicinity of the forest.

2011, Tanizaki et al. 2013) as well as in Europe, Oceania and North America [for review, see Müller and Schlagenhauf (2014)]. To our knowledge, there is no record of *P. knowlesi* cases in Africa or Central and South America.

Ta et al. (2014) described, also in Malaysia, the first case of naturally acquired human infection by *Plasmodium cynomolgi*, which naturally infects old world monkeys in Africa and Southeast Asia. *P. cynomolgi* is morphologically indistinguishable from *P. vivax* and the molecular diagnostic protocol most commonly used worldwide (Snounou et al. 1993) cannot distinguish between *P. vivax* and *P. cynomolgi* DNA, possibly because the 18s rRNA gene is conserved between these species. Therefore, other diagnostic protocols that are able to amplify another target are required for correct identification of this parasite (Ta et al. 2014).

Therefore, there is now a consensus that some species of *Plasmodium* that typically infect non-human primates such as *P. cynomolgi*, *Plasmodium simiovale*, *P. knowlesi*, *Plasmodium inui* and *Plasmodium eylesi* in Asia, *Plasmodium schwetzi* in Africa and *P. brasilianum* and *P. simium* in the New World, may, under special conditions, infect humans (Deane et al. 1966, Deane 1992, Ta et al. 2014).

In the tropical and subtropical New World, the natural hosts of simian plasmodia are *Alouatta* spp, *Ateles* spp, *Brachyteles arachnoides*, *Cacajao* spp, *Callicebus* spp, *Cebus* spp, *Chiropotes* spp, *Lagothrix* spp, *Saimiri* spp, *Saguinus midas* and *Pithec*ia spp for *P. brasilianum* and *Allouatta fusca* and *B. arachnoides* for *P. simium* (Deane 1992, Lourenço-de-Oliveira & Deane 1995, Fandeur et al. 2000). Both New World non-human primate malaria parasites were originally described in animals from Brazil: the quartan malaria parasite *P. brasilianum* was described in an Amazonian monkey (*Cacajao calvus*) exposed in a circus in Antwerp (Gonder & Berenberg-Gossler 1908) and the tertian *P. simium* was described from a blood smear of an *A. fusca* examined during sylvatic yellow fever studies in Itapecerica, SP (da Fonseca 1951). Plasmodial infection in non-human wild primates in Brazil was comprehensively studied from the 1930s to the 1990s by Leônidas de Mello Deane, who examined more than 4,000 animals, showing that 14.3% were infected and the geographical distribution and prevalence varied considerably throughout the country; non-human primates from the southeastern (35.6%) and southern (17.9%) regions exhibit the highest prevalence. *P. simium* is restricted to these two regions, while *P. brasilianum* is spread widely throughout South America (i.e., Brazil, Panama, Venezuela, Colombia and Peru) (Lourenço-de-Oliveira & Deane 1995) and is the only species found in the Brazilian Amazon. Considering Brazil as a whole, *P. brasilianum* is the most prevalent in infected animals (found in ~74% of positive blood films) and *P. brasilianum* and *P. simium* account for 46.3% and 37.5% of the infections in the Southeast and for 33.3% and 42.4% of the infections in the South Region, respectively. Simian malaria was absent in the Northeast and West-Central regions and mixed infections may account for close to 20% of the infections in meridional areas (Deane 1992). It is worth noting that the prevalence of plasmodial infection may be high among non-human primates in Brazil; this is the case in the howler monkey *A. fusca* from SP, in which the prevalence reaches 53.2% even when only blood film examination is considered (Deane 1992). In addition, a high proportion of monkeys has been found to carry antibodies against blood forms and sporozoites or DNA of *P. brasilianum* and *P. simium*, suggesting that the prevalence of plasmodial infection may be much higher than previously expected (Deane 1992, Curado et al. 2006, Duarte et al. 2008, Yamasaki et al. 2011). Although there is a substantial lack of data on the prevalence of simian malaria throughout the country, it may be holoenzootic in certain areas, such as southeastern Brazil.

It has ever been noted that New World simian plasmodia are very closely related to human plasmodia; the blood forms of *P. simium* and *P. brasilianum* are morphologically identical to those of *P. vivax* and *P. malariae*, respectively (Deane 1992). The advent of molecular and genetic approaches has further elucidated the co-identity of these parasites. Goldman et al. (1993) suggested that *P. simium* could be a strain of *P. vivax*. Escalante et al. (1995, 2005) demonstrated the genetic identity between *P. vivax* and *P. simium* and between *P. brasilianum* and *P. malariae* based on an analysis of the conserved regions of the gene coding for the circumsporozoite surface protein (CSP) and suggested that the cross transmission of plasmodia between humans and New World monkeys occurred recently on the evolutionary scale. Lim et al. (2005) studied two strains of *P. simium* of Brazilian origin and showed that their CSP gene sequences are genetically indistinguishable from those of 24 strains of *P. vivax*. Genetic blurring of the two species was confirmed at 13 microsatellite *loci* and eight tandem repeats. The data also indicated that the transfer between humans and monkeys must have occurred twice because both variants (i.e., VK210 and VK247) are present in tandem repeats in both *P. vivax* and *P. simium* species (Lim et al. 2005). Concerning *P. brasilianum,* it has been demonstrated that monoclonal antibodies against the CSP of this parasite cross-react with those for *P. malariae* and strong molecular similarities have been described in these species (Cochrane et al. 1985, Escalante et al. 1998, Yamasaki et al. 2011). Accordingly, several authors have suggested host transference of *P. simium* vs. *P. vivax* and *P. brasilianum* vs. *P. malariae,* although the direction remains unknown (Tazi & Ayala 2011, Guimarães et al. 2012).

Therefore, the presence of wild monkeys carrying *P. vivax/P. simium* and *P. malariae/P. brasilianum* or monkeys carrying antibodies against blood forms and/or sporozoites of *P. malariae* and *P. vivax* and their variants in the Atlantic Forest suggests that monkeys that live in these areas can act as a reservoir for human infections and may be responsible for the maintenance of foci in areas under the influence of this biome. Such a possibility has not been formally confirmed by the studies conducted to date in the affected areas. These data indicate the urgent need to further investigate the possibility of malaria as a zoonosis, primarily in areas where *Anopheles (K.) cruzii* participates in transmission (i.e., in the Atlantic Forest of the Southeast Brazil). Work currently being undertaken by Brazilian teams in areas of the Atlantic Forest may help to clarify some of these questions.

In conclusion, there is a high receptivity and vulnerability of regions outside of the Amazon due to the presence of competent malaria vectors and a suitable climate and environment for malaria transmission in almost all of the extra-Amazonian region. Large and frequent human movements have occurred in recent years between the Amazonian and extra-Amazonian regions. Therefore, a sensitive and perennial surveillance system is required for the early detection of malaria cases to provide immediate treatment and to prevent local transmission and deaths in extra-Amazonian Brazil. In addition, as the extra-Amazonian region area has annually reported imported cases from African countries, optimal and rapid diagnostics are essential for the appropriate management of the disease to prevent severe manifestations of malaria, including deaths caused largely by *P. falciparum*.

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